

CLAIMS

What is claimed is:

5 1. A method of inducing proliferation of isolated human marrow stromal cells in vitro, the method comprising

 providing the isolated cells and a growth medium to a growth surface such that the initial density of the isolated cells is less than about 50 cells per square centimeter of growth surface, and

10 incubating the surface under growth-promoting conditions, whereby the cells proliferate.

 2. The method of claim 1, wherein the initial density of the isolated cells is less than about 25 cells per square centimeter of growth surface.

15 3. The method of claim 1, wherein the initial density of the cells is less than about 12 cells per square centimeter of growth surface.

20 4. The method of claim 1, wherein the initial density of the cells is less than about 10 cells per square centimeter of growth surface.

 5. The method of claim 1, wherein the initial density of the cells is less than about 6 cells per square centimeter of growth surface.

25 6. The method of claim 1, wherein the initial density of the cells is less than about 3 cells per square centimeter of growth surface.

 7. The method of claim 1, wherein the initial density of the cells is less than about 1.5 cells per square centimeter of growth surface.

30 8. The method of claim 1, wherein the initial density of the cells is less than about 1.0 cells per square centimeter of growth surface.

9. The method of claim 1, wherein the initial density of the cells is at least about 0.5 cells per square centimeter of growth surface.

10. The method of claim 1, wherein the cells are harvested from the growth surface following not more than about 14 days of incubation.

11. The method of claim 1, wherein the cells are harvested from the growth surface following not more than about 10 days of incubation.

12. The method of claim 1, wherein harvested cells and a growth medium are provided to a second growth surface such that the initial density of the harvested cells is less than about 50 cells per square centimeter of second growth surface and the second growth surface is incubated under growth-promoting conditions, whereby the cells proliferate.

13. The method of claim 12, wherein the cells are seeded on the growth surface at an initial density of about 3 cells per square centimeter.

14. The method of claim 12, wherein the cells are harvested from the second growth surface following not more than about 14 days of incubation.

15. The method of claim 12, wherein the cells are harvested from the second growth surface following not more than about 10 days incubation.

16. The method of claim 12, wherein cells harvested from the second growth surface and a growth medium are provided to a third growth surface such that the initial density of the cells harvested from the second growth surface is less than about 50 cells per square centimeter of third growth surface and the third growth surface is incubated under growth-promoting conditions, whereby the cells proliferate.

17. The method of claim 16, wherein the cells are harvested from the third growth surface following not more than about 14 days of incubation.

18. The method of claim 16, wherein the cells are harvested following not more than about 10 days of incubation.

19. The method of claim 16, wherein the cells are seeded on the growth surface at an initial density of about 3 cells per square centimeter.

20. The method of claim 1, wherein the growth medium comprises a mammalian serum.

21. The method of claim 20, wherein the mammalian serum is fetal bovine serum.

22. The method of claim 1, wherein a growth factor is added to the growth medium.

23. The method of claim 22, wherein the growth factor is selected from the group consisting of fibroblast growth factor, platelet derived growth factor, insulin growth factor, and endothelial growth factor.

24. A method of enhancing *in vitro* proliferation of isolated human marrow stromal cells growing on a surface in the presence of a growth medium, the method comprising supplementing the growth medium with a factor present in a conditioned medium, wherein the conditioned medium is obtained from a culture of producer human marrow stromal cells which are grown on a second surface at an initial density of at least about 0.5 cells per square centimeter and which are incubated for at least about 3 days.

25. The method of claim 24, wherein the producer human marrow stromal cells are grown on the second surface at an initial density of at least about 12 cells per square centimeter.

26. The method of claim 24, wherein the producer human marrow stromal cells are incubated for at least about 6 days.

27. The method of claim 24, wherein the growth medium is supplemented with the factor by supplementing the growth medium with the conditioned medium.

28. The method of claim 24, wherein the growth medium is supplemented with the factor by size-fractionating the conditioned medium and then supplementing the growth medium with a fraction of the conditioned medium containing size-fractionated molecules having a molecular weight of about 30,000.

29. The method of claim 24, wherein the growth medium is supplemented with the factor by size-fractionating the conditioned medium and then supplementing the growth medium with a fraction of the conditioned medium containing size-fractionated molecules having a molecular weight of about 10,000.

30. A conditioned medium for inducing proliferation of human marrow stromal cells, wherein the conditioned medium is made by incubating human marrow stromal cells for at least about 3 days on a surface in the presence of a growth medium at an initial density of less than about 12 cells per square centimeter of surface, whereby the growth medium is transformed into conditioned medium.

31. A method of inducing proliferation of human marrow stromal cells, the method comprising isolating mononuclear cells from a bone marrow sample, incubating the mononuclear cells to yield colonies, isolating an individual colony, and incubating human marrow stromal cells obtained from the isolated colony in a container having a growth surface, the container containing a growth medium and the cells at an initial density of less than about 50 cells per square centimeter of growth surface, whereby the cells proliferate.

32. A method of assessing the expandability of human marrow stromal cells *in vitro*, the method comprising incubating the cells on a surface in the presence of a growth medium at an initial density of less than about 50 cells per square centimeter of surface and assessing the colony-forming efficiency of the cells, whereby the expandability of the cells is approximately proportional to the colony-forming efficiency of the cells.

33. The method of claim 32, wherein the cells are incubated for at least about 10 days.

34. The method of claim 32, wherein the colony-forming efficiency is compared with the colony-forming efficiency of another sample of human marrow stromal cells incubated in the same manner, wherein the expandability of the cells of the other sample is known.

35. The method of claim 32, wherein the colony-forming efficiency is compared with a reference plot of colony-forming efficiency versus expandability

36. The method of claim 35, wherein the plot is Figure 2.

37. A method of isolating marrow stromal cells having a greater expansion potential from marrow stromal cells having a lesser expansion potential, the method comprising separating a population of marrow stromal cells into a first fraction having a size less than about 10 micrometers and second fraction having a size greater than about 10 micrometers, whereby cells of the first fraction have a greater expansion potential than cells of the second fraction.

38. A method of providing an expression construct to an animal, the method comprising

- i) isolating marrow stromal cells from an animal of the same species
- ii) thereafter
 - a) electroporating the isolated marrow stromal cells in the presence of the expression construct to generate transformed isolated marrow stromal cells and
 - b) expanding the isolated marrow stromal cells *in vitro* and
- iii) thereafter providing the transformed isolated marrow stromal cells to the animal, whereby the expression construct is provided to the animal.

39. The method of claim 35, wherein the marrow stromal cells are isolated from the same animal.

40. A method of purifying marrow stromal cells, the method comprising contacting a population of stromal cells with an antibody to Lipocortin II, and isolating from the population of stromal cell cells which bind the antibody, thereby purifying the marrow stromal cells.

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41. A method of purifying marrow stromal cells, the method comprising providing isolated marrow stromal cells and a growth medium to a growth surface such that the initial density of the isolated cells is less than about 50 cells per square centimeter of growth surface,

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incubating the surface under growth-promoting conditions, whereby the cells proliferate,

contacting the population of stromal cells with an antibody to Lipocortin II, and isolating from the population of stromal cell cells which bind the antibody, thereby purifying the marrow stromal cells.